JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Synthesis, Biological Evaluation, and Molecular Docking Studies of New Nitro Vanillin Analogues as Anti-glycating Agents

Sajjad Anjum[1], Priya Tufail[2], Sajjad Haider[2], Taibi Ben Hadda[3], Asad Ullah[1], Sabira Begum[1], Humera Jahan[2], Zaheer Ul-Haq[2], Bina Shaheen Siddiqui[1]*

[1]H.E.J Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan.
[2]Dr. Panjawani Center for Molecular Medicine and Drug Development, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan.
[3]Laboratoire de Chimie des Matériaux, Faculté des Sciences, Université Mohammed Premier,60000 Oujda, Morocco.

Abstract: Persistent hyperglycemia is linked to a range of chronic complications in diabetes, such as neuropathy, retinopathy, nephropathy, and atherosclerosis. The underlying cause is the highly stable advanced glycation end products (AGEs) resulting from prolonged exposure to high glucose level. Hence the present study was undertaken on the anti-glycation activity of a series of synthetic analogues (Schiff bases) **4a-4o** of nitrovanillin synthesized by its coupling with different amino reagents. Nitrovanillin was obtained by the nitration of vanillin. Vanillin is a natural product that was obtained by the reduction of vanillic acid. Vanillic acid is another natural product which was isolated from ethanol extract of plant species *Tamarix aphylla* during the current study. These analogues were screened for *in-vitro* anti-glycation activity using rutin (IC_{50} = 180±0.8 μM) as a reference molecule. The best potent analogues **4a** (IC_{50} = 121±1.0 μM), **4f** (IC_{50} = 95.0±0.7 μM), and **4h** (IC_{50} = 183±3.8 μM) were subjected to computational study that revealed they were not only anti-glycation active, but also having well in ligand–protein interaction profile. While, all others analogues were found moderate to highly active. When the safety profile of these analogues **4a-4o** was evaluated by MTT assay using HepG2 cells against doxorubicin as a reference drug, the analogues **4a**, **4e**, **4f**, **4i**, **4l**, **4m**, and **4o** were found nontoxic, while analogues **4d**, **4h, 4k**, and **4n** showed insignificant toxicity.

Keywords: AGEs, Amadori product, anti-glycation, Column chromatography, Docking study, *Tamarix aphylla*.

Submitted: January 4, 2024. **Accepted:** June 3, 2024.

Cite this: Anjum S, Tufail P, Heider S, Ben Hadda T, Ullah A, Begum S, Jahan H, Ul-Haq Z, Siddiqui BS. Synthesis, Biological Evaluation, and Molecular Docking Studies of New Nitro Vanillin Analogues as Anti-glycating Agents. JOTCSA. 2024;11(3): 1255-66.

DOI: https://doi.org/10.18596/jotcsa.1402128

***Corresponding author's E-mail:** siddiqui_bina@yahoo.com

1 INTRODUCTION

Glycation is a non-enzymatic spontaneous process initiated when reducing sugars react with biological molecules such as amino acids, lipids, and nucleic acids. Glycation results in the formation of unstable Schiff bases when an excess amount of free blood sugars i.e., glucose, ribose, and fructose react with the free amino group proteins in the living system which then undergoes (1) several modifications through Amadori rearrangement and ends up with the generation of very stable advanced glycation end products (AGEs) (2, 3). In hyperglycemic conditions, excessive formation of AGEs causes various chronic complications of diabetes mellitus (4), a very frequent heterogeneous disorder that affected almost 415 million people worldwide. The situation is so alarming that, by the end of 2040 the number will be increased to 642 million (5). Other diabetes-linked complications due to AGEs are tumor malignancy (6), nephropathy, neuropathy, retinopathy, atherosclerosis, and stroke (7). Certain structural distortions and malfunctions were also observed due to the glycation of blood albumins which trigger the formation of reactive oxygen species as well as prevent their scavenging capability for free radicals and create oxidative stress (8). The formation of AGEs is a normal process of body

metabolism (9) but an accelerated rate under hyperglycemia in tissues and circulation level promotes pathogenic complications and inflammatory response (10-12). Glycation of biological molecules in the living system not only involves AGEs generation but, dietary food items also act as a source of these species. They naturally exist in uncooked animal-derived foods and their contamination further increased during cooking at high temperatures to enhance flavor, color, and appearance. The fact that modern food items are the richest sources of AGEs is now well-documented since it was previously assumed that foods born AGE are poorly absorbed and their adverse health effects were ignored (13, 14). The destructive effects of AGEs paid great attention to the prevention of the formation of these species and the execution of these effects. The discovery of synthetic as well as natural anti-glycation agents with minimum side effects and high efficiencies such as flavonoid, phenol derivatives, imidazole, thiazolidine, and sulfonate were employed to control AGEs-linked complications (15). Although numerous anti-glycation agents have been developed in the past few years they have not gained attraction to prevent the process of glycation and manage the effects of AGEs such as aminoguanidine, which was not approved for clinical use due to toxicity and its adverse effects (16, 17). Similarly some safe drugs approved by FDA (USA) such as metformin, aspirin, diclofenac etc., but not so effective to prevent glycation during hyperglycemic conditions. However, some anti-glycating agents ie, ALT-711, benfotiamine, *etc* are under investigation for this aspect (18). Hence, there is a need to investigate safe and effective ant-glycation agents to treat glycation-associated disorders. Schiff bases have gained the overwhelming attraction of researchers due to their ubiquitous behavior in the field of medicine and pharmaceutics due to anti-bacterial (19, 20) anti-tumor (21), anti-fungal (22), and anti-proliferative activities (23). Through our continuous effort to search the Schiff bases as anti-glycation agents, were reported the substituted synthesized benzenediol Schiff bases which were significantly active as AGEs inhibitors to cure diabetes-associated complications (24). With this motivation during the current study, a series of analogues **4a-4o** (Schiff bases) of nitro vanillin (Scheme-2) were synthesized and evaluated against the non-enzymatic glycation of protein using methylglyoxal-bovine serum albumin (MGO-BSA) glycation model. Their cytotoxicity evaluation was also conducted by employing an MTT assay using HepG2 (a human liver cancer cell line) cells. The analogues which showed the best anti-glycation activity were subjected to computational study to identify the possible binding sites of legend and protein targets by selecting the acarbose as a reference legend. Nitrovanillin was prepared by nitration (25) of vanillin **2** (Scheme-1): which was obtained by conversion of vanillic acid **1** (26) (Scheme-1). Vanillic acid was isolated during the present study from the ethanol extract of aerial parts of the plant *Tamarix aphylla.* The synthesis of these nitro vanillin derivatives, their *in-vitro* anti-glycation study, and molecular docking was conducted for the first time. The analogues reported in this communication were new except **4a** (27) and showed significant anti-glycation effects without any cytotoxicity on the MTT assay.

2. EXPERIMENTAL SECTION

2.1. General Consideration

All analytical grade reagents were purchased from Sigma Aldrich USA *Jahan et., al.* Protocol (US9387198) (28) and were used to prepare AGES. Phosphate buffer reagents were purchased from Duksan Pure Chemicals Co. Ltd. (NMR spectra at 400 MHz) and were recorded on a Bruker AM spectrometer in DMSO-d_6 with a residual peak of dimethyl sulfoxide (δ = 2.50 ppm ^{1}H, 39.5 ppm, ^{13}C). Chemical shifts were reported in parts per million (ppm) relative to TMS (δ). Coupling constants were recorded to the nearest 0.1 Hz. Signal multiplicity was reported as singlet (s), doublet (d), triplet (t) quartet (q), double doublet (dd), and multiplet (m).^{13}C spectra were recorded on an advanced Bruker 75 MHz spectrometer chemical shift recorded in parts per million (ppm). EI-MS spectra were recorded on MAT113D and MAT 312 mass spectrometers; Melting points were recorded on Buchi melting points-560 apparatus. The pre-coated silica gell-254 Merck Germany plates were used for thin-layer chromatography to monitor the reaction progress UV lights at 366 and 254 nm were used to visualize the spots. Normal phase column chromatography was conducted for the isolation of vanillic acid.

2.2. Isolation of Vanillic Acid 1

Chromatographic techniques were employed on silica gel to isolate the sufficient amount of compound **1** (vanillic acid) from aerial parts extract of plant species *Tamarix aphylla.* The compound **1** was then converted into nitro vanillin **4**. The nitro vanillin was used to synthesize the series of Schiff bases **4a-4o**.

2.3. General Procedure for Synthesis of Compounds 2-4

Compound **1**, 10 mmol was treated with an equal amount of DIBEL-H followed by cooling at -10 °C and stringing for 1 hour in THF to afford compound **2** with 93% yield (26) (scheme-1). The compound **2**, 8 mmol was, reflux, with anequal amount of MnO_2, in THF for 3 hour and compound **3** was obtained with 93% yield (26) (Scheme-1). Compound **3**, 7.2 mmol was refluxed with fuming HNO_3 at 60 °C for 1hour in MeCN and afforded compound **4** (nitro vanillin) with a 95% yield (25) (Scheme-1).

*2.3.1. General procedure for synthesisofanalogues **4a-4o***

The analogues **4a-4o** is derivatives of compound **4**. Compound **4** was refluxed with different amino reagents in 10 mL anhydrous ethanol at 70-80 °C for 4-5 hours in the presence of the catalytic amount of glacial acetic acid followed by the reported protocol (29). Tabel-1 (Scheme-2). When each reaction was completed the precipitate appeared which was filtered, washed with distilled water, and recrystallized with ethanol. The percent yield of all analogues was calculated.

2.3.2. *(E)-2-(4-Hydroxy-3-methoxy-5-nitrobenzylidene)hydrazine-1-carbothioamide (**4a**)*
Yellow solid, yield 55%, m.p.: 179-180 °C, ^{1}H NMR (DMSO-d_6, 400 MHz); δ_H 10.95(s, 1H, OH), 7.86 (1H, brs, NH_2), 7.78 (1H, s, H-1′), 7.71 (1H, brs, NH2), 7.39 (1H, d, *J* = 2.0 Hz, H-6), 7.07 (1H, d, *J* = 2.0 Hz, H-2), 3.89 (s, 3H, OCH_3). EI-MS m/z (% rel. abund.): 270 (M^+), 253 (8), 236 (8), 194 (100), 177 (81), 164 (17), 121 (21), 104 (16), 90 (22), 59 (21), 44 (17). HREI-MS calcd for $C_9H_{10}N_4O_4S$: m/z = 270.0423 found 270.0427

*2.3.3. (E)-2-(4-Hydroxy-3-methoxy-5-nitrobenzylidene) hydrazine-1-carboxamide (**4b**)*
Yellow solid, yield 54 %, m.p.: 134-137 °C ^{1}HNMR (DMSO-d_6, 400 MHz): δ_H 7.76 (1H, s, H-1′), 7.67 (1H, s, H-2), 7.66.9 (1H, s, H-6), 7.50 (s, 1H, NH), 6.58 (s, 2H, NH_2), 23.92 (3H, s, OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 159.7 (C), 150.5 (C), 144.2 (CH), 136.9 (C), 130.3 (C) 125.7 (C), 121.76 (C), 123.1 (CH), 111.2 (CH), 55.8 (CH3). EI-MS m/z (% rel. abund.): 254 (M^+, 85), 237 (19), 211 (11), 194 (100), 181 (22), 135 (83), 120 (30), 105 (10), 78 (12), 61 (52), 53 (18), 44 (7). HREI-MS calcd $C_9H_{10}N_4O_5$: m/z = 254.0651 found 254.0657

*2.3.4. (2,4-Dinitrophenyl)hydrazineylidene)methyl)-2-methoxy-6-nitrophenol (**4c**)*
Yellow powder, yield 55 %, m.p.: 185-188 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 8.87 (1H, d, *J* = 2.4 Hz, H-2), 8.50 (1H, s, H-1′), 8.35 (1H, dd, *J* = 9.0 and 2.0, Hz, H-5″), 8.10 (1H, d, *J* = 9.0 Hz, H-6″), 7.71 (1H, s, H-3″), 7.29 (1H, brs, H-6), 3.76 (3H, s, OCH_3). 13C NMR (DMSO-d_6, 100 MHz): δ_C 153.9 (C), 150.0 (C), 149.2 (C), 144.0 (C), 136.2 (C), 135.3 (C) 129.5 (C), 128.7 (CH), 123.1 (CH), 121.2 (CH), 116.7 (CH), 107.7 (CH), 106.7 (CH), 55.8 (CH3). EI-MS m/z (% rel. abund.): 377 (M^+, 100), 347 (14), 315 (14), 296 (8), 285 (5), 269 (9), 253 (4), 239 (4), 223 (4) 197 (35), 180 (19), 152 (14), 122 (12), 106 (6), 79 (11), 63 (13), 44 (10). HREI-MS calcd $C_9H_{10}N_4O_5$: m/z = 254.0651 found 254.0657

*2.3.5. (E)-2-Methoxy-6-nitro-4-((2-phenylhydrazineylidene) methyl)phenol (**4d**)*
Yellow solid, yield 55%, m.p.: 39-41 °C, ^{1}H NMR (DMSO-*d*6, 400 MHz): δ_H 7.79 (1H, s, H-1′), 7.62 (1H, s, *J* = 2.0 Hz, H-2), 7.55 (d, 1H, *J* = 2.0 Hz, H-6), 7.22 (2H, t, *J* = 8.0 Hz, H-3″and H-5″), 7.07 (2H, d, *J* = 7.6 Hz, H-6″and, H-2″), 6.75 (1H, t, *J* = 7.2 Hz, H-4″), 3.93 (3H s, , OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 149.7 (CH), 147.3 (C), 145.1 (C), 142.4 (C), 137.2 (C), 134.5 (C), 129.0 (CH) 126.8 (CH), 118.8 (CH), 115.0 (CH), 113.5 (CH). 112.0 (CH), 111.5 (CH), 56.5(CH_3), 121.2, 116.7, 107.6, 55.8; EI-MS EI-MS m/z (% rel. abund.): 287 (M^+, 100), 252 (7), 238 (2), 225 (6), 209 (4), 184 (2),169 (2), 143 (3), 133 (2) 107 (1), 92 (38), 77 (9), 65 (9), 50(2) HREI-MS calcd r $C_9H_{10}N_4O_5$: m/z = 254.0651 found 254.0657.

*2.3.6. (E)-2-Methoxy-6-nitr(((4(trifluoromethyl)phenyl)imino)methyl)phenol (**4e**)*
Yellow solid, yield 50%, m.p.: 90-93 °C, ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 7.98 (1H, s, H-1′), 7.80 (2H, d, *J* = 8.2 Hz, H-5″ and H-3), 7.69 (2H, d, *J* = 2.0 Hz, H-6 and H-2), 7.59 (2H, d, *J* = 8.0 Hz, H-6″ and H-2″), 3.94 (3H, s, OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 159.4 (C), 149.7 (C), 146.4 (C), 145.1 (C), 142.3 (C), 137.2 (C) 134.5 (CH), 129.0 (CH), 126.8 (CH). 118.8 (CH), 113.5(CH), 112.0 (CH), 111.5(CH), 56.5 (CH_3), EI-MS m/z (% rel. abund.): 340 (M^+, 100), 321 (7), 310 (3), 293 (30), 279 (3), 264 (12), 251 (9), 248 (6), 235 (2) 222 (7), 202 (1), 196 (2), 172 (7), 153 (2), 145 (17), 125 (2), 107 (1), 95 (3), 79 (1), 75 (2), 63 (1), 53 (1), 51(1). HREI-MS calcd $C_{15}H_{11}F_3N_2O_4$:m/z = 340.0908 Found 340.0904.

*2.3.7. (E)-2-Methoxy-6-nitro-4-(((2-(trifluoromethyl)phenyl) imino)methyl)phenol (**4f**)*
Yellow solid, yield 55 %, m.p.: 103-106 °C , ^{1}H NMR (DMSO-d_6 400 MHz): δ_H 8.64 (1H, s, H-1′), 8.08 (1H, s, H-2), 7.92 (1H, brs, H-6), 7.26 (1H, d, *J* = 6.0 Hz, H-3″), 7.11 (1H, t, *J* = 6.0 Hz, H-4″), 6.91 (1H, d, *J* = 6.0 Hz, H-6″), 6.85(1H, t, *J* = 6.0 Hz, H-5″), 3.98 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): δc 160.2(CH), 157.3 (C), 155.0 (C), 150.2 (C),147.0 (C), 144.0 (C), 136.3 (C),135.3 (C), 129.5 (CH), 128.7 (CH), 123.1 (CH), 121.2 (CH), 116.7 (CH), 107.6 (CH), 55.8 (CH_3): EI-MS m/z (% rel. abund.): 340 (M^+), 293 (1), 197 (100), 180 (53), 152 (7), 149 (20), 135 (24), 248 (6), 235 (2) 222 (7), 202 (1), 196 (2), 172 (7), 122 (9), 108 (3), 93 (3), 79,(14), 65 (11), 51 (10), 41 (1). HREI-MS calcd $C_{15}H_{11}F_3N_2O_4$: m/z = 340.0671 Found 340.0677.

2.3.8. (E)-2-Methoxy-6-nitro-4-(((3-(trifluoromethyl)phenyl)imino)methyl)phenol (**4g**)
Yellow solid, yield 52%, m.p.: 91-94 °C, ^{1}HNMR (DMSO-d_6, 400 MHz): δ_H 8.68 (1H, s, H-1′), 8.08(2H, d, *J* = 2.0 Hz, H-6 and H-2), 7.68 (1H, t, *J* = 8.0 Hz, H-5″), 7.60(2H, d, *J* = 8.0 Hz, H-6″and, H-4″), 7.56 (1H, s, H-2″), 3.92 (3H, s, OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 154.4 (CH), 151.7 (C), 150.4 (C), 144.0 (C), 140.8 (C), 136.2 (C) 135.3 (CH), 129.5 (C), 128.8 (C). 1124.8 (CH), 124.9 (CH), 123.1 (CH), 121.2(CH), 116.7 (CH), 56.5 (CH_3), EI-MS m/z (% rel. abund.): 340 (M^+, 100), 321 (6), 293 (26), 264 (10), 251 (5), 222 (7), 200 (2), 172 (7), 145 (19), 95 (2), 75 (2), 51 (2). HREI-MS calcd for $C_{15}H_{11}F_3N_2O_4$: m/z = 340.0671 Found 340.0675.

*2.3.7. (1E, 1'E)-Hydrazine-1,2-diylidenebis(methaneylylidene))bis(2-methoxy-6-nitrophenol) (**4h**)*
Red solid, yield 56%, m.p.: 177-179 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 8.48 (2H, s, H-1′, H-1″) 7.79 (2H, s, H-2, H-2‴), 7.35 (2H, s, H-6, H-6‴), 3.80 (6H, s, OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 190.1 (CH), 150.5(C), 149.2(C), 136.9 (C), 125.7 (C), 121.6 (CH), 111.7 (CH), 56.2(CH_3). EI-MS m/z (% rel.abund.): 390 (M^+, 100), 373 (7), 360 (9), 312 (3), 295 (1), 267 (2), 239 (1), 225 (1), 222 (31) 196 (4), 17 (5), 176 (6), 164 (2), 149 (4), 135 (8), 106 (2.5), 92 (2), 78 (2), 53 (2), 44 (2). HREI-MS calcd for $C_{16}H_{14}N_4O_8$: m/z = 390.0812 Found 390.0818.

*2.3.9. (E)-2-methoxy-6-nitro-4-((phenylimino) methyl) phenol (**4i**)*
Yellow solid, yield 55%, m.p.: 42-45 °C,^{1}HNMR (DMSO-d_6, 100 MHz,): δH 8.60 (1H, s, H-1′), 8.02

(1H, s, H-2), 7.75 (IH, s, H-6), 7.43 (2H, d, *J* = 7.0 Hz, H-1″, H-6″), 7.30 (3H, t, *J* = 8.0 Hz, H-3″, H-4″ H-5″), 3.92 (3H, s, OCH_3). ^{13}C NMR (DMSO-d_6, 400 MHz): δ_C 158.6 (CH), 149.7 (C), 145.1 (C), 142.0 (C), 133.6 (C), 129.0 (C) 125.3 (C), 118.8 (CH), 128.8 (CH). 113.5 (CH), 112.9 (CH), 111.7 (CH), 111.5 (CH), 111.2(CH), 56.8 (CH_3), EI-MS m/z (% rel, abund): 272 (M^+, 100), 242 (3), 196 (9), 183 (8), 154 (5), 127 (3), 77 (13), 51 (3). HREI-MS calcd for $C_{14}H_{12}N_2O_4$: m/z = 272.0797 Found 272.0793.

*2.3.10. (E)-2-(4-Hydroxy-5-methoxy-3-nitrobenzylideneamino)benzoic acid (**4j**)*

White solid, yield 51 %, mp.: 175-178 °C , ^{1}HNMR (DMSO-d_6, 400 MHz): δ_H 8.69 (1H, s, H-1′), 8.08 (2H, d, *J* = 1.2 Hz, H-2 and H-6), 7.83 (1H, t, *J* = 6.0 Hz, H-5″), 7.1 5 (1H, brs, H-2″), 7.11 (1H, m, H-4″), 6.76 (1H, dd, *J* = 8.0, 2.0 Hz, H-6″) 3.92 (3H, s, OCH_3). 13C NMR (DMSO-d_6, 100 MHz): δC 163.45 (CH), 160.1 (CH), 149.7 (C), 145.1 (C), 142.4 (C), 137.2 (C), 134.5 (C) 129.0 (C), 126.8 (C), 123.4 (CH). 118.8 (CH), 113.5 (CH), 112.0 (CH), 111.5(CH), 56.5 (CH_3), EI-MS m/z (% rel. abund.): 316 (M^+, 100), 286 (74), 242 (5), 225 (8), 197 (18), 167 (8), 111 (11), 97 (21), 92 (40), 77 (14), 69 (19) 44 (37). HREI-MS calcd $C_{15}H_{12}N_2O_6$: m/z = 316.0695. Found 316.0692.

*2.3.11. (E)-4-(((2-Hydroxyphenyl)imino)methyl)-2-methoxy-6-nitrophenol (**4k**)*

White solid, yield 54%, m.p.: 173-176 °C, ^{1}HNMR (DMSO-d_6, 400 MHz): δ_H 8.79 (1H, s, H-1′), 8.23 (1H, s, H-2), 8.13 (1H, s, H-6), 7.42 (1H, d, *J* = 6.0 Hz, H-6″), 7.25 (1H, t, *J* = 7.5 Hz, H-4″), 7.07 (1H, d, *J* = 8.0 Hz, H-6″), 7.02 (1H, t, *J* = 6.0 Hz, H-5″), 4.09 (3H, s, OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 159.2 (CH), 154.5 (C), 150 0 (C), 144.0 (C), 140.5 (C), 136.2 (C), 135.3 (C) 129.5 (CH), 128.7 (CH), 123.1 (CH). 121.28 (CH), 116.7 (CH), 107.6.0 (CH),), 55.8 (CH3), EI-MS m/z (% rel. abund): 288 (M^+, 100) 241 (12), 225 (5), 212 (3), 195 (2), 169 (2), 154 (1), 144 (2), 127 (1), 93 (3), 85 (1), 65 (5), 43 (1). HREI-MS calcd $C_{14}H_{12}N_2O_5$: m/z = 288.0746 Found: 288.0740.

*2.3.12. (E)-2-Methoxy-4-(((5-(methylthio)-2-(trifluoromethyl)phenyl)imino)methyl)-6-nitrophenol (**4l**)*

White solid, yield 51%, m.p.: 139-14 °C, ^{1}HNMR (DMSO-d_6, 400 MHz): δ_H 8.75 (1H, s, H-1′), 8.20 (2H, d, *J* = 2.0, Hz, H-2 and H-6), 7.87 (1H, s, H-6″), 7.71 (2H, d, *J* = 8 .0 Hz, H-4″and H-3″), 4.07 (s, 3H, OCH_3), 3.98 (s, 3H, SCH3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 159.2 (CH), 154.5.1 (C), 150.0 (C), 144.0 (C), 140.5 (C), 136.2 (C), 135.3 (C), 129.5 (C), 128.7 (C), 123.1 (C), 121.2 (CH), 116.7 (CH), 107.6 (CH), 111.5 (CH), 55.8 (CH_3), 14.6 (CH_3). EI-MS m/z (% rel. abund.): 386 (M^+ 100) 371 (17), 353 (52), 307 (13), 290 (1), 278.2 (2), 25 (1), 222 (1), 218 (9), 185 (2), 157 (2) 133 (1), 63 (1). HREI-MS calcd $C_{16}H_{13}F_3N_2O_4S$: *m/z* = 386.0548 Found 386.0542.

*2.3.13. (E)-4-Hydroxy-3-methoxy-5-nitrobenzaldehyde oxime (**4m**)*

White powder, yield 55%, m.p.: 102-104 °C, ^{1}HNMR (DMSO-d_6, 400 MHz): δ_H 8.13 (1H, s, H-1′), 7.66 (d, 1H, *J* = 2.0 Hz, H-2), 7.45 (d, 1H, *J* = 2.0 Hz, H-6), 3.88 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 149.6 (CH), 146.5 (C), 143.4 (C), 136.9 (C), 123.7 (C), 114.8 (CH), 112.0 (CH), 56.4 (CH3). EI-MS m/z (% rel, abund): 212 (M^+, 100), 195 (22), 177 (6), 152 (6), 139 (11), 108 (7), 96 (4), 77 (7), 63 (4), 53 (8). HREI-MS calcd for $C_8H_8N_2O_5$: *m/z* = 212.0433 Found 212.0437

*2.3.14. (E)-4-(((3,4-Dichlorophenyl)imino)methyl)-2-methoxy-6-nitrophenol (**4n**)*

White powder, yield 55%, m.p.: 102-104 °C, ^{1}HNMR (DMSO-d_6, 400 MHz): δ_H 8.13 (1H, s, H-1′), 7.66 (d, 1H, *J* = 2.0 Hz, H-2), 7.45 (d, 1H, *J* = 2.0 Hz, H-6), 3.88 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 149.6 (CH), 146.5 (C), 143.4 (C), 136.9 (C), 123.7 (C), 114.8 (CH), 112.0 (CH), 56.4 (CH3). EI-MS m/z (% rel, abund): 212 (M^+, 100) 195 (22), 177 (6), 152 (6), 139 (11), 108 (7), 96 (4), 77 (7), 63 (4), 53 (8).EI-MS *m/z* (% rel. abund.): 240 (M^+,100), 242 (M^{+2}, 64), 244 (M^{+4}, 11), 323 (4), 310 (15), 293 (17), 280 (3), 268 (6), 266 (5), 251 (5), 222 (2), 187 (3), 172 (4), 145 (7), 109 (4), 79 (1), 63 (1), 51 (1), HREI-MS calcd $C_{14}H_{10}Cl_2N_2O4$: *m/z* = 340.0018 Found 340.0012.

*2.3.15. (E)-2-Hydroxy-5-((4-hydroxy-3-methoxy-5-nitrobenzylidene)amino acid **(4o)***

White solid: yield:54%, m.p.:196-199 °C, ^{1}HNMR (DMSO-d_6, 400 MHz,): δ_H 8.67 (1H, s, H-1'), 8.08 (2H, d, *J* = 2.0 Hz, H-2 and H-6), 7.77 (1H, d, *J* = 6.0 Hz, H-6″), 7.56 (1H, d, *J* = 6.0 Hz, H-5″), 6.92 (1H, d, *J* = 8.0, Hz, H-6″), 7.18 (1H, brs, H-2″) 3.90 (3H, s, OCH_3). 13C NMR (DMSO-d_6, 100 MHz): δ_C 160.1 (CH), 154.5 (C), 152.6 (C), 150.0 (C), 144.0 (C), 136.2 (C), 135.3 (C), 129.5 (C), 128.7 (CH), 123.1 (CH), 121.2 (CH), 116.7 (CH), 107.6 (CH), 56.4 (CH3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 168.5 (C), 160.1 (CH), 149.7 (C), 145.1 (C), 142.4 (C), 137.2 (C), 134.5 (C), 129.0 (C), 126.8 (CH), 123.4 (CH), 118.8 (CH), 116.7 (CH), 113.5 (CH), 112.0 (CH), 111.5 (CH) 56.5 (CH3). EI-MS *m/z* (% rel. abund.): 332 (M^+, 36), 288 (100), 258 (57), 241 (16), 197 (10), 153 (7), 135 (12), 109 (18), 93 (7), 79 (10), 65 (10), 53 (7). HREI-MS calcd $C_{15}H_{12}N_2O_7$: *m/z* = 332.0645 Found 332.0649.

2.4. Anti-glycation Study

2.4.1. Assay for anti-glycation study

The AGES were prepared according to the optimized protocol (US9387198) (27) briefly, disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4) (Duksan Pure Chemicals Co. Ltd.) were used to prepare a 100 mM phosphate buffer solution. Bovine serum albumin (BSA= 10 mg/mL) (Sigma Aldrich) and methylglyoxal (MGO 500 mM) (Thermo Fisher Scientific) were mixed with azide buffer (0.1 mM). All the working dilutions were prepared using deionized water. All the compounds were dissolved in dimethyl sulfoxide (DMSO) (Amresco LLC). The assay was performed in a flat-bottom 96-well black fluorescence plate (Corning Inc.). Initially, the nitro vanillin analogues were evaluated at 1mM concentration. Each compound was tested in triplicates. Rutin hydrate (1 mM) (Sigma Aldrich) was used as a reference glycating agent. BSA mixed with

sodium phosphate buffer was used as a negative control. The reaction plate was kept at 37 °C for 24 hours for incubation. The anti-glycation potential of all analogues was analyzed by measuring AGEs specific fluorescence (355 nm excitation and 460 nm emission) against blank by using Varioskan Lux microtitre plate reader (Thermo Fisher Scientific). Percent (%) inhibition of AGEs was calculated by using the formula given below

Inhibition of fluorescence % = (1-Fluorescence of test derivative / Fluorescence of glycated BSA) x 100

The compounds exhibiting anti-glycation potential at 1 mM (> 50% inhibition) were further diluted 2 fold and their IC_{50} values were determined by using the EZ-FIT Enzyme Kinetics protocol (Perrella Scientific Inc).

2.4.2. Cytotoxicity assay

HepG2 a human liver cancer cell line was purchased from ATCC (USA) and maintained in a sterile environment. Initially, the cells were cultivated in a 25 cm^2 cell-culture flask (Nest Co.Ltd.). The cell culture medium was prepared with Dulbecco's Modified Eagle Medium-high glucose (DMEM), fetal bovine serum (FBS), sodium pyruvate, sodium bicarbonate, and L-Glutamine (Gibco). The cells were kept in an incubator at 37 °C providing 5% CO_2 until they become 80% confluent. Later, the cells were trypsinized using 1X trypsin-EDTA (0.25%) and viability was determined using trypan blue. HepG2, $8x10^4$ cells / mL, were seeded in a 96-well flat-bottom sterile cell culture plate and incubated at 37 °C for 24 hours for adherent. The cells were treated with the analogues at 50 μM concentration in triplicates for 24 hours. The medium was aspirated and 100 μL MTT dye (5 mg/mL) was added to the cells. After 3 hours, the dye was removed, and 10% DMSO was added to each well to dissolve the formazan crystals. Colorimetric analysis was performed at 570 nm, using a spectrophotometer (Varioskan micro plate reader Thermo Fisher). The toxic effect of analogues on cell viability was measured by the formula given below.

Inhibition % =100 – [(Absorbance of test Compound-Absorbance of blank) / (Absorbance of control–Absorbance of blank)]

2.4.3. Structure activity relationship of analogues ***4a-4o***

A series of nitro vanillin analogues **4a-4o** were prepared and tested for anti-glycation assay. All analogues have a common parent methoxy nitro phenol ring and variable region. The analogues were classified into three categories based on the basis of presences of different functional groups in the variable region for the study of the structure-activity relationship. Category "A" included analogues **4a** and **4b**, which bear the carbamide functional group, category "B" include analogues **4c** and **4d** which bear the hydrazinelidene functional group, and category "C" **4e-4o** included imino functional group other than the parent ring in their structure as shown in Table.1 The compounds exhibited excellent to moderate anti-glycation potential against the *in-vitro* MGO-modified BSA model. The IC_{50} values were found in the range of 95 to 465 μM. Rutin was used as standard which showed 62% inhibition in MGO-BSA glycation assay with IC_{50} = 180±0.8 μM). Nitro vanillin analogues showed weak (glycation inhibition percentage < 50%), moderate (glycation inhibition percentage >50% and <70%), good (glycation inhibition percentage >70%), and excellent (glycation inhibition percentage >80%) anti-glycation activity. Analogue **4a** (81.83% inhibition, IC_{50} = 121±1.0 μM) showed excellent anti-glycation activity compared to the reference rutin (62% inhibition, IC_{50} = 180±0.8 μM). Rutin is a flavonoid, known for inhibiting early steps in the glycation process and hence modulates the formation of AGES. The activity of analogue **4a** may be attributed to hydrazine thiocarbamide moiety in its structure. Analogue **4b** (48.33% inhibition) is structurally similar to analogue **4a** but it contains hydrazine carboxamide moiety and showed poor anti-glycation activity less than 50% inhibition compared to rutin and considered inactive. From the results, it is inferred that the replacement of thiocarbamide with carbamide moiety caused a compound inactive.

The analogues **4c** and **4d** contain phenyl hydrazine group and exhibited poor to good anti-glycation activity. analogue **4c** (44.31% inhibition) bears two nitro groups at *ortho* and *para* positions of phenyl hydrazinylidene ring in its structure resulting in less than 50 % inhibition and ranked as anti-glycation in active. The presence of the unsubstituted phenyhydrazenylidene group in analogue **4d** (69.71% inhibition, IC_{50} = 220±0.15 μM), showed good anti-glycationactivity. The comparison between these two analogues reveals that either presence of two nitro groups at *ortho* or *para* position compromise analogue **4c** inactive or the presence of an un-substituted phenyl hydrazine ring boosts up the potency of analogue **4d** against the glycation process. The analogues **4a-4o** bears phenyl imino moieties with substituted or unsubstituted phenyl amino ring in their structures and showed variation in activity from compound to compound. Thus, the presence of the tri-fluoromethane group at different positions of the phenylamino ring in some of these analogues revealed excellent to weak anti-glycation activity. The presence of this group at the *para* position resulted in moderate activity in analogue **4e** (80.82% inhibition, IC_{50} = 336±2.9 μM), but the presence of this group at the *ortho* position in analogue **4f** (79.62% inhibition, IC_{50} = 95.0 ± 0.7 μM) boosts up the activity and so that it behaves as a strongest anti-glycation candidate than all the synthetic analogues of series as it exhibited excellent anti-glycation activity.

On the other hand, the presence of the tri-fluoro methane group at the *meta* position of the phenylamino ring turns down the activity of analogue **4g** (45.30% inhibition) towards the weakest candidate so that, it showed less than 50% inhibition and stands as an inactive anti-glycating agent. Analogue **4h** (74.24% inhibition, IC_{50} = 183±3.8 μM), a dimer of the parent compound showed

excellent anti-glycation activity comparable with that of rutin. The anti-glycation activity of this compound may be attributed to the presence of either a di-methylidene hydrazine group or two aromatic rings. Analogue **4i** (56.92% inhibition, IC_{50} = 331±1.1 μM) is without substitution on its phenylimino ring and showed moderate anti-glycation activity. Its activity could be compared with analogue **4j** and **4k**. Analogue **4i** is more active than analogue **4j** (42.10% inhibition) which showed less than 50% inhibition and which bears the acidic group at the *meta* position of its phenyl imino ring in its structure and is biologically inactive while analogue **4i** is less active than analogue **4k** (59.89 % inhibition, IC_{50} =237.0±2.2 μM) which bears hydroxy group at the *ortho* position of phenylimino ring and showed moderate anti-glycation activity compared to standard rutin. These results indicated that the presence of a hydroxy group at the *ortho* position of the phenylimino ring enhances the activity while the presence of an acidic group at the *meta* position causes the analogue to be inactive. Analogue **4l** (70.64% inhibition, IC_{50} = 187±0.6 μM) bears a tri-fluoro methane group at the *ortho position* and thiomethane group at the *meta* position of phenylamino ring, when the activity of this analogue is compared with the activity of the analogue **4f**, which has only a trifluoromethane group at *ortho* position and which is a most potent compound of series, it is observed that presence of thiomethane group at *meta* position reduces the activity of analogue **4l** however its activity is still greater than analogue **9** which have tri-fluoro methane group at the *para* position and analogue **4g** which have tri-fluoro methane group at *meta* position respectively. These results also revealed that the presence of thiomethame at the *meta* position along with tri-fluoromethane at the *ortho* position of the phenyl imino ring reduces the anti-glycation activity. The analogue **4m** (61.16% inhibition and IC_{50} = 361±10.0 μM) bears an oxime moiety and exhibited moderate anti-glycation activity compared to rutin. This analogue differs from other members of the series as it bears only the parent phenyl ring that is common to all analogs, so its activity cannot be compared with any other analogue in the series. The analogue **4n** (71.18% inhibition IC_{50} = 214±2.4 μM), bears two chlorine groups at *meta* and *para* positions of the phenylimino ring and showed good anti-glycation activity. The activity of this analogue iscompared to the activity of analogue **4o** (55.64% inhibition, IC_{50} = 245±2.4 μM) which bears ahydroxy group at *meta* and acidic group at *para* positions and also showed good anti-glycation activity compared to rutin but less active than analogue **4n**.These results indicated that the substitution of phenyl imino ring at *ortho* and *meta* positions with chlorine groups enhances the activity while activity decreases when these positions are substituted with an acidic group at *ortho* and a hydroxy group at the *meta* position. The results are shown in Table 1. The safety profile of the analogue was evaluated by MTT assay using HepG2 cells. The analogue found with weak anti-glycation activity was not assessed for cytotoxicity. Analogue **4a**, **4e**, **4f**, **4i**, **4l**,**4m**, and **4o** were found nontoxic at 50 μM concentration when compared to the standard drug, doxorubicin, whereas analogue **4d**, **4h**, **4k,** and **4n** were fairly toxic as compared to standard doxorubicin at 50 μM. The results are sown in Table 1.

Table1: *In-vitro* anti-glycation study and cytotoxicity evaluation of nitro vanillin analogous **4a-4o**.

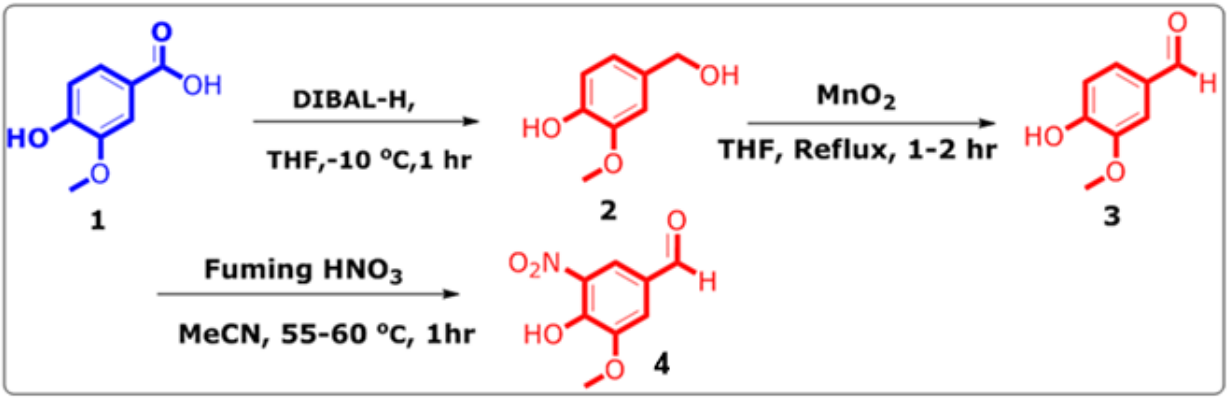
Analogues	Anti-glycation Inhibition %	Anti-glycation IC_{50} (μM ± SEM)	Cytotoxic Inhibition % 50 μM
4a	81.83	121±1.0	16.49
4b	NA	NA	NA
4c	NA	NA	NA
4d	69.71	220±1.5	47.88
4e	82.82	336±2.9	21.13
4f	79.62	95±0.7	0.10
4g	NA	NA	NA
4h	74.24	183±3.8	57.32
4i	56.92	331±1.1	2.39
4j	NA	NA	NA
4k	59.89	237±2.2	46.67
4l	70.64	187±0.6	16.8
4m	61.16	361±1.0	7.34
4n	71.18	214±2.4	32.32
4o	55.64	245±2.4	0.20
Doxorubicin	62.23	180±0.8	
Rutin			45.98±2.42

NA = INACTIVE

3. RESULTS AND DISCUSION

3.1. Chemistry

The primary approach in our study was rested to select the smaller non-toxic, and biological active nucleus so that its new activity is investigated by the preparation of its synthetic analogues. Since natural products are less or non toxic and possess several biological activities so our study rounds about natural products. To fulfill our approach, we conducted column chromatography on the methanolic extract of aerial parts of the plant species *Tamarix aphylla* and isolated a non-toxic natural product vanillic acid **1** which is ubiquitous in several biological activities. To make this molecule more versatile it was transformed *via* three steps reactions into an aldehyde motif nitro vanillin. The first step of transformation was a reduction of vanillic acid **1** into alcoholic moiety **2** which by oxidation in the second step converted into vanillin **3.** Vanillin **3** bears the aldehyde functional group susceptible to amino moiety for hydrazone synthesis. Since its *para* position is covered by the methoxy group which due to the positive mesomeric effect pretends the carbonyl group towards less susceptible to incoming moiety and yield was not so good. To enhance the yield vanillin was subjected to nitration and converted into nitro vanillin compound **4** (Scheme-1).



Scheme 1: Conversion of vanillic acid **1** into nitro vanillin **4**.

Compound **4** was utilized as an intermediate for the generation of a series of Schiff bases **4a-4o,** with excellent yield, Table-2 (Scheme-2). Our second approach was to evaluate the anti-glycation activity and bio-safety and establish the structure-activity relationship of these analogues (Table-1). Our third approach was a molecular docking study of the most potent analogues (Figure-2) and (Figure-3).

3.2. Structure Elucidation of Most Active Analog 4f

3.2.1. ^{1}H and ^{13}C-NMR of analog 4f

The ^{1}H NMR of most potent analouge **4f** was recorded on a Bruker AM (DMSO-d_6, 400 MHz). In the spectrum of this compound the most down field signal was the signal of proton H-1' resonating at δ 8.64 as a singlet. The proton H-2 resonated as a singlet at δ 8.08 was the second down filed proton. Then there was a singlet signal of proton H-6 resonated at δ 7.29 Proton H-3'' showed up as a doublet at δ 7.26 with a coupling constant (J = 6.0 Hz). Furthermore, there was a triplet of proton H-4'' resonating at δ 7.33. A doublet signal of proton H-6'' appeared at δ 6.91 (J = 6.0 Hz). The proton H-5'' resonated at δ 6.85 as triplet. The most up field signal was the signal of methoxy protons resonating as a singlet at δ 3.98.

^{13}C-NMR spectrum of the analog **4f** showed signals including seven quaternary signals signals resonating at δ_C 157.3, 155.0, 150.2, 147.0, 144.0, 136.3 and 135.3, while seven methene carbons were resonating at at δc 160.2, 129.5 128.7, 123.1, 121.2, 116.7 and 107.6. A most upfield methyl carbon signal appeared at δc 55.8. This profile confirms the expected structure of analogue **4f** Figure-1.

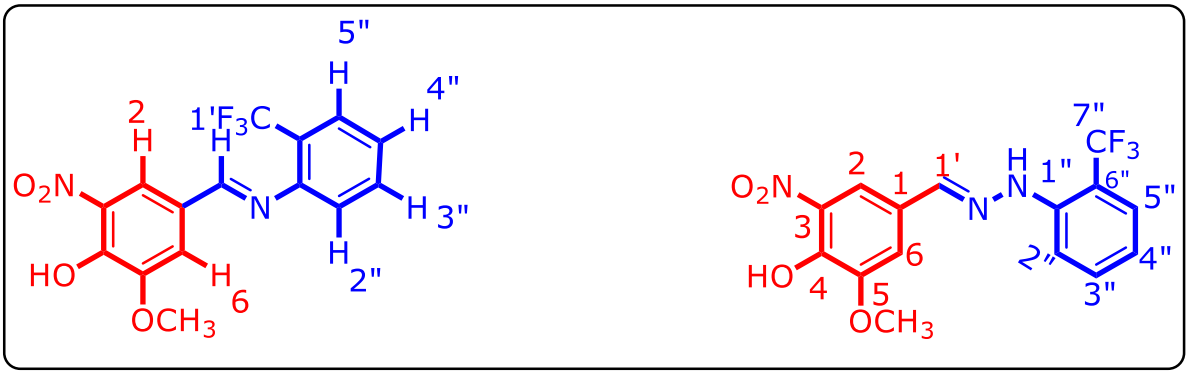
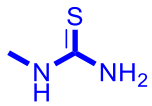
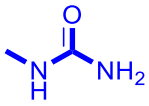
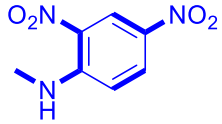
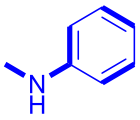
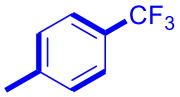
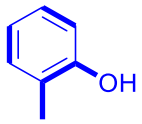
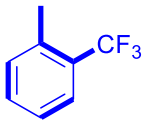
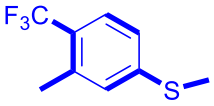
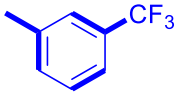
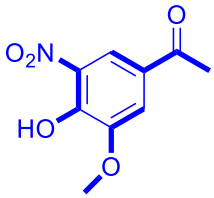
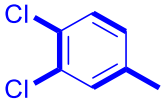
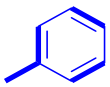
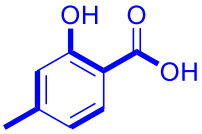
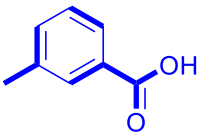
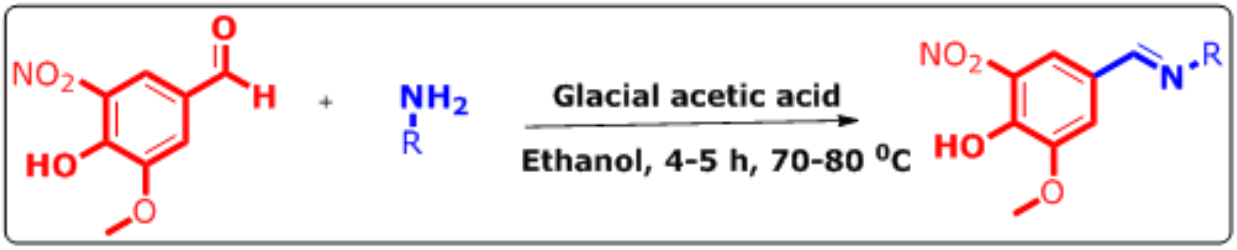


Figure 1: ^{1}H and ^{13}C-NMR of analogue **4f**.

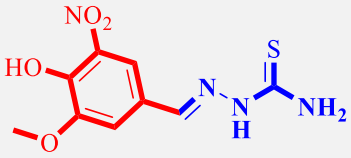
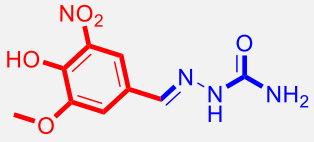
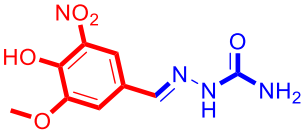
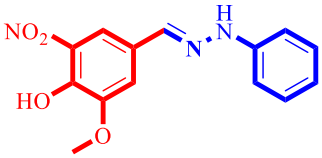
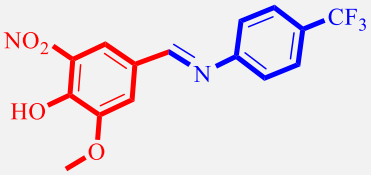
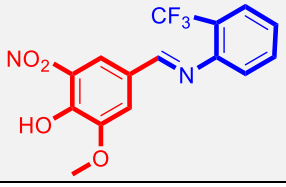
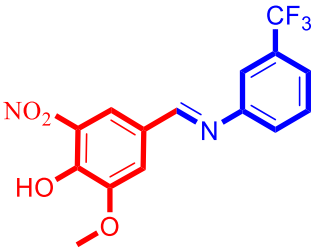
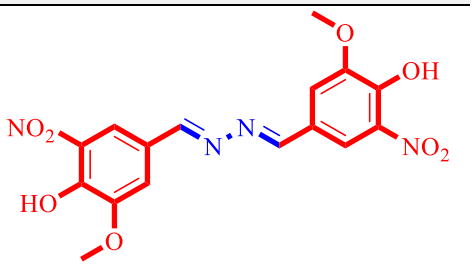
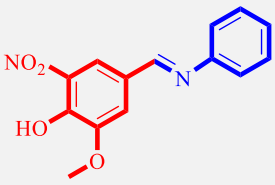
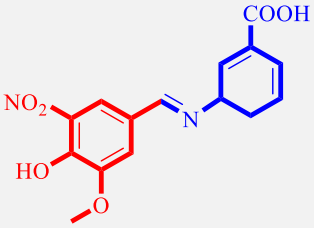
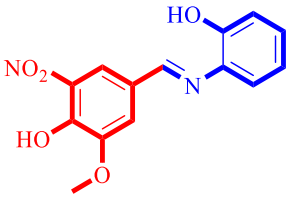
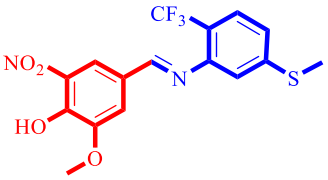
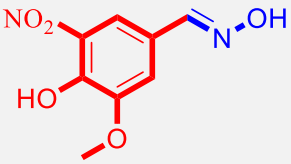
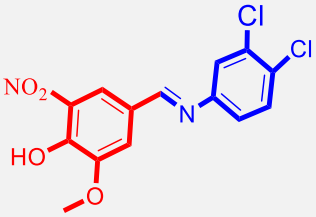
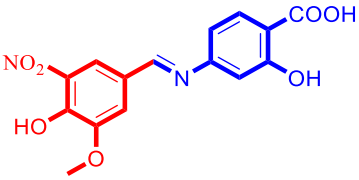
Table 2: Synthesized nitro vanillin analogues **4a-4o**.

Entry	R	Analogues	Entry	R	Analogues
		Category "A"			
1		**4a**	2		**4b**
		Category "B"			
3		**4c**	4		**4d**
		Category "C"			
5		**4e**	11		**4k**
6		**4f**	12		**4l**
7		**4g**	13		**4m**
8		**4h**	14		**4n**
9		**4i**	15		**4o**
10		**4j**			



Scheme 2: Synthetic analogues of nitro vanillin **4a-4o**

Table 3: Synthetic analogs **4a-4o**.

Analog	Structure	Analog	Structure
4a		**4b**	
4c		**4d**	
4e		**4f**	
4g		**4h**	
4i		**4j**	
4k		**4l**	
4m		**4n**	
4o			

4. MOLECULAR DOCKING STUDY

Molecular docking is a computational and standard technique for identifying a binding mechanism of ligands and protein targets (30). The docking program implemented in the Molecular Operating Environment 2019.01 was used with default parameters to explore the binding mode of the synthesized vanillin derivatives. The 3D structures of all the synthetic compounds were model, protonated and energy minimized using the MMFF94 force field implemented in MOE 2019.01(31, 32) conformations were generated for each compound and the best-ranked pose was selected for further study. Furthermore, all the docking poses were visualized

and all the images of the docked poses were prepared through the UCSF Chimera program (33).

4.1. Results and Discussion

Molecular docking is a well-known and most promising tool that provides the possible binding mechanisms between protein and ligand. In this study, docking protocol was applied on a series of vanillin derivatives to explore interactive mechanism with *Saccharomyces cerevisiae*. Previously reported α-glucosidase homology model template (PDB ID: 3A, 4A) has been used (34). The vanillin derivatives and comparative antagonist acarbose were docked into the binding pocket of the receptor by using the default operating system of MOE. The protein ligand interactions of the docked pose of each complex were visualized manually. The highest ranked dock score conformer of vanillin derivatives and reference ligand (acarbose) were selected. The Docking score of the synthetic compounds having the best anti-glycation activity such as analogues **4a**, **4f**, **4h** and reference ligand was-6.91, -6.99, -7.96 and -4.382 kcal/mol respectively. The docking result revealed that acarbose as well as all the synthetic compounds binds in the binding pocket of the α-glucosidase (Table-4). Assemble binding pose of all the analogs along with reference ligand in α-glucosidase are illustrated in (Figure-3).

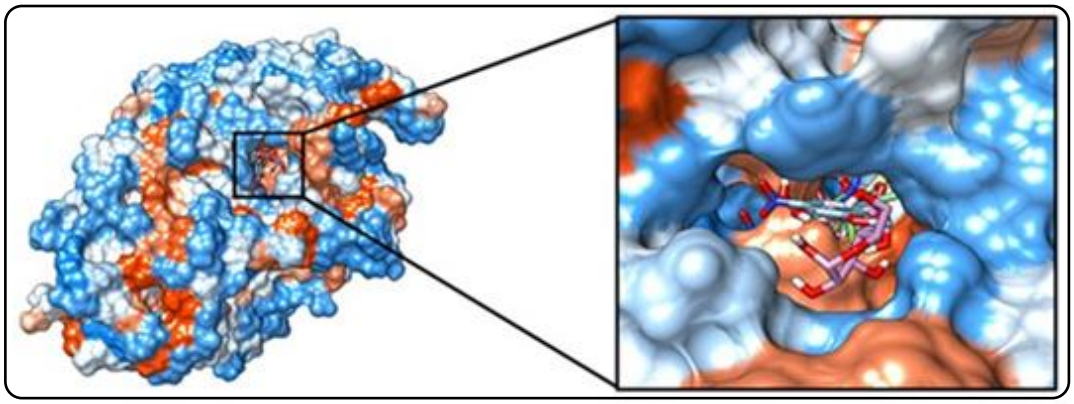


Figure 2: The assemble dock pose of Vanillin analogues **4a**, **4f**, **4h**, and reference ligand (acarbose) against the binding pocket of α-glucosidase are depicted in the 3D format.

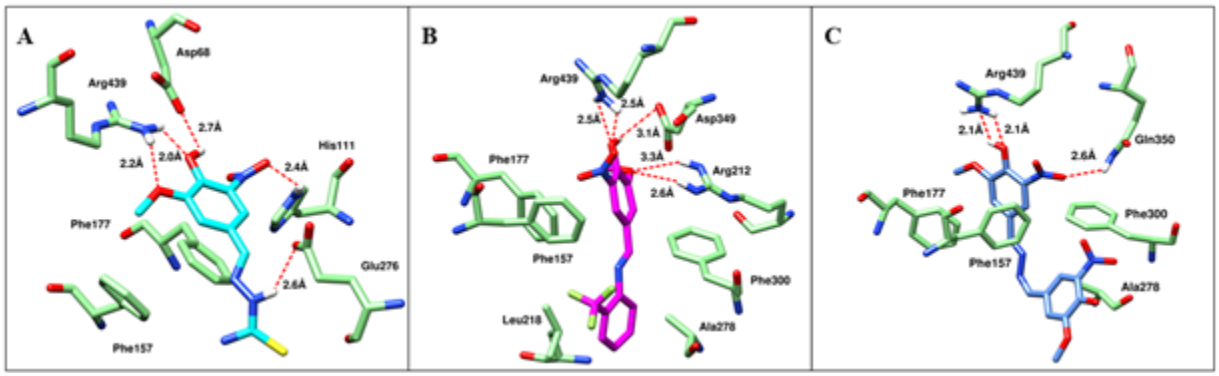


Figure 3: 3D graphical structure representing the binding interaction of analogous **4a**, **4f**, and **4h** (A, B, C) in the active pocket of α-glucosidase.

Table 4: Molecular interactions residue and binding energy of docked compounds in the binding pocket of *α*-glucosidase.

Compound ID	Binding Energy Kcal/mol	H-Bond	Hydrophobic	Pi-Stacking
Reference compound	-4.382	Arg212, His111, Arg439	Glu276, Glu304, Asp349	Phe177, His239, Pro309
analog **4a**	-6.91	Asp68, Arg439,His111, Glu276	Phe157	Arg439, Phe177
analog **4f**	-6.99	Arg439, Asp349Arg212	Phe157, Phe177, Leu218, Ala278, Phe300	----
analog **4h**	-7.96	Arg439, Gln350	Phe157, Phe177, Ala278, Phe300	----

5. CONCLUSION

In the current study, the analogues **4a-4o** were synthesized on the core nucleolus of nitro vanillin by coupling reaction of different amino reagents on the carbonyl group of nitro vanillin in an acid medium followed by a Schiff base mechanism. The analogues were subjected to *in-vitro* screening against AGES through an *in-vitro* MGO-modified BSA model by using rutin as a standard compound. Besides analogues **4b**, **4c**, **4g,** and **4j** which were inactive, other analogues were moderate to highly active. Among potent analogues, the best active analogue was analogue **4f** which bears the trifluoromethyl group at the phenyl imino ring in its structure. The other highly active analogs were analogue **4a**, which bears the carbothioamide group, analogue **4h** which bears the hydrazine group, and analogue **4l** which bears 5-methylthio-2-trifluoromethyl phenylimino group. While analogue **4d**, **4e**, **4i**, **4k 4m, 4n,** and **4o** were considered moderately active. The structure-activity relationship study revealed that the presence of the carboxamide group in analogue **4b**, the presence of the 2,4-dinitrophenyl group in analogue **4c**, the presence of trifluoromethyl group at *meta* position in the molecular structure of analogue **4g**, and the presence of an acidic group at *meta* position in the molecular structure of analogue **4j** reduced the activity of these compounds so lower that they are ranked as inactive against AGES in the series. When safety measures of the analogs **4a-4o** were conducted by MTT against HePG2 cell lines their safety profile reveals that analogs **4a**, **4e**, **4f**, **4i**, **4l**, **4m**, and **4o** were nontoxic concerning doxorubicin as a standard drug, Analogues **4d**, **4h**, **4k** and **4n** were found fairly toxic while inactive analogues were not subjected in this study. The molecular docking study conducted on the best potent analogues, **4a**, **4f,** and **4h** showed excellent results. From these results we can infer that our approaches to preparing the synthetic analogues of the smaller, natural, and relatively non-toxic molecule are successful. The analogues are significantly active against AGEs and non toxic and can be used as lead molecules in the future for the treatment of diabetic-associated complications. The docking study of these analogs reveals that they were not potent only in *in-vitro* but also best in legend-protein based interaction as well.

6. CONFLICT OF INTREST

Authors declare no conflict of interest.

7. REFERENCES

1. Ahmad S, Moinuddin, Dixit K, Shahab U, Alam K, Ali A. Genotoxicity and immunogenicity of DNA-advanced glycation end products formed by methylglyoxal and lysine in presence of Cu^{2+}. Biochem Biophys Res Commun [Internet]. 2011 Apr 15;407(3):568–74. Available from: <URL>.

2. Twarda-Clapa A, Olczak A, Białkowska AM, Koziołkiewicz M. Advanced Glycation End-Products (AGEs): Formation, Chemistry, Classification, Receptors, and Diseases Related to AGEs. Cells [Internet]. 2022 Apr 12;11(8):1312. Available from: <URL>.

3. Lin J, Wu C, Lu C, Hsia S, Yen G. Glycative stress from advanced glycation end products (AGEs) and dicarbonyls: An emerging biological factor in cancer onset and progression. Mol Nutr Food Res [Internet]. 2016 Aug 25;60(8):1850–64. Available from: <URL>.

4. Peppa M, Vlassara H. Advanced glycation end products and diabetic complications: A General overview. Hormones [Internet]. 2005;4(1):28–37. Available from: <URL>.

5. International diabetes federation. Diabetes Atlas [Internet]. Brussels: Belgium: International Diabetes Federation; 2015. Available from: <URL>.

6. Yeh WJ, Hsia SM, Lee WH, Wu CH. Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. J Food Drug Anal [Internet]. 2017 Jan 1;25(1):84–92. Available from: <URL>.

7. Arya SS, Rookes JE, Cahill DM, Lenka SK. Vanillin: a review on the therapeutic prospects of a popular flavouring molecule. Adv Tradit Med [Internet]. 2021 Sep 7;21(3):415–33. Available from: <URL>.

8. Cade WT. Diabetes-Related Microvascular and Macrovascular Diseases in the Physical Therapy Setting. Phys Ther [Internet]. 2008 Nov 1;88(11):1322–35. Available from: <URL>.

9. Rondeau P, Bourdon E. The glycation of albumin: Structural and functional impacts. Biochimie [Internet]. 2011 Apr 1;93(4):645–58. Available from: <URL>.

10. Eble AS, Thorpe SR, Baynes JW. Nonenzymatic glucosylation and glucose-dependent cross-linking of protein. J Biol Chem [Internet]. 1983 Aug 10;258(15):9406–12. Available from: <URL>.

11. Schmidt AM, Yan S Du, Wautier JL, Stern D. Activation of Receptor for Advanced Glycation End Products. Circ Res [Internet]. 1999 Mar 19;84(5):489–97. Available from: <URL>.

12. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract [Internet]. 2014 Feb 1;103(2):137–49. Available from: <URL>.

13. Vlassara H, Uribarri J. Glycoxidation and Diabetic Complications: Modern Lessons and a Warning? Rev Endocr Metab Disord [Internet]. 2004 Aug;5(3):181–8. Available from: <URL>.

14. O'Brien J, Morrissey PA, Ames JM. Nutritional and toxicological aspects of the Maillard browning reaction in foods. Crit Rev Food Sci Nutr [Internet]. 1989 Jan 1;28(3):211–48. Available from: <URL>.

15. Abbas G, Al-Harrasi AS, Hussain H, Hussain J, Rashid R, Choudhary MI. Antiglycation therapy: Discovery of promising antiglycation agents for the

management of diabetic complications. Pharm Biol [Internet]. 2016 Feb 8;54(2):198–206. Available from: <URL>.

16. Ahmad S, Shahab U, Baig MH, Khan MS, Khan MS, Srivastava AK, et al. Inhibitory Effect of Metformin and Pyridoxamine in the Formation of Early, Intermediate and Advanced Glycation End-Products. Agarwal PK, editor. PLoS One [Internet]. 2013 Sep 4;8(9):e72128. Available from: <URL>.

17. Ahmad S, Khan MS, Akhter F, Khan MS, Khan A, Ashraf JM, et al. Glycoxidation of biological macromolecules: A critical approach to halt the menace of glycation. Glycobiology [Internet]. 2014 Nov 1;24(11):979–90. Available from: <URL>.

18. Goh SY, Jasik M, Cooper ME. Agents in development for the treatment of diabetic nephropathy. Expert Opin Emerg Drugs [Internet]. 2008 Sep 2;13(3):447–63. Available from: <URL>.

19. da Silva CM, da Silva DL, Modolo L V., Alves RB, de Resende MA, Martins CVB, et al. Schiff bases: A short review of their antimicrobial activities. J Adv Res [Internet]. 2011 Jan 1;2(1):1–8. Available from: <URL>.

20. Neelakantan M, Esakkiammal M, Mariappan S, Dharmaraja J, Jeyakumar T. Synthesis, Characterization and Biocidal Activities of Some Schiff Base Metal Complexes. Indian J Pharm Sci [Internet]. 2010 Feb 1;72(2):216–22. Available from: <URL>.

21. Liang C, Xia J, Lei D, Li X, Yao Q, Gao J. Synthesis, in vitro and in vivo antitumor activity of symmetrical bis-Schiff base derivatives of isatin. Eur J Med Chem [Internet]. 2014 Mar 3;74:742–50. Available from: <URL>.

22. Fugu MB, Ndahi NP, Paul BB, Mustapha AN. Synthesis, characterization, and antimicrobial studies of some vanillin schiff base metal (II) complexes. Available online www.jocpr.com J Chem Pharm Res [Internet]. 2013;5(4):22–8. Available from: <URL>.

23. Song WJ, Cheng JP, Jiang DH, Guo L, Cai MF, Yang HB, et al. Synthesis, interaction with DNA and antiproliferative activities of two novel Cu(II) complexes with Schiff base of benzimidazole. Spectrochim Acta Part A Mol Biomol Spectrosc [Internet]. 2014 Mar 5;121:70–6. Available from: <URL>.

24. Jhaumeer-Laulloo S, Gupta Bhowon M, Mungur S, Fawzi Mahomoodally M, Hussein Subratty A. In vitro Anti-glycation and Anti-oxidant Properties of Synthesized Schiff Bases. Med Chem (Los Angeles) [Internet]. 2012;8(3):409–14. Available from: <URL>.

25. Rakshit S, Lakshminarasimhan T, Guturi S, Kanagavel K, Kanusu UR, Niyogi AG, et al. Nitration Using Fuming HNO 3 in Sulfolane: Synthesis of 6-Nitrovanillin in Flow Mode. Org Process Res Dev [Internet]. 2018 Mar 16;22(3):391–8. Available from: <URL>.

26. Ribeiro TS, Freire-de-Lima L, Previato JO, Mendonça-Previato L, Heise N, Freire de Lima ME. Toxic effects of natural piperine and its derivatives on epimastigotes and amastigotes of Trypanosoma cruzi. Bioorg Med Chem Lett [Internet]. 2004 Jul 5;14(13):3555–8. Available from: <URL>.

27. Shah MS, Rahman MM, Islam MD, Al-Macktuf A, Ahmed JU, Nishino H, et al. Synthesis, antimicrobial and antioxidant evaluation with in silico studies of new thiazole Schiff base derivatives. J Mol Struct [Internet]. 2022 Jan 15;1248:131465. Available from: <URL>.

28. Jahan H, Siddiqui NN, Iqbal S, Basha FZ, Shaikh S, Pizzi M, et al. Suppression of COX-2/PGE2 levels by carbazole-linked triazoles via modulating methylglyoxal-AGEs and glucose-AGEs – induced ROS/NF-κB signaling in monocytes. Cell Signal [Internet]. 2022 Sep 1;97:110372. Available from: <URL>.

29. Naganagowda G, Meijboom R, Petsom A. Synthesis and Antimicrobial Activity of New Schiff Base Compounds Containing 2-Hydroxy-4-pentadecylbenzaldehyde Moiety. Adv Chem [Internet]. 2014 Jul 21;2014(1):1–9. Available from: <URL>.

30. Ul-Haq Z, Khan A, Ashraf S, Morales-Bayuelo A. Quantum mechanics and 3D-QSAR studies on thienopyridine analogues: inhibitors of IKKβ. Heliyon [Internet]. 2020 Jun 1;6(6):e04125. Available from: <URL>.

31. Halgren TA. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. J Comput Chem [Internet]. 1996 Apr;17(5–6):490–519. Available from: <URL>.

32. Haider S, Barakat A, Ul-Haq Z. Discovery of Potential Chemical Probe as Inhibitors of CXCL12 Using Ligand-Based Virtual Screening and Molecular Dynamic Simulation. Molecules [Internet]. 2020 Oct 20;25(20):4829. Available from: <URL>.

33. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera—A visualization system for exploratory research and analysis. J Comput Chem [Internet]. 2004 Oct 1;25(13):1605–12. Available from: <URL>.

34. Ali M, Barakat A, El-Faham A, Al-Rasheed HH, Dahlous K, Al-Majid AM, et al. Synthesis and characterisation of thiobarbituric acid enamine derivatives, and evaluation of their α-glucosidase inhibitory and anti-glycation activity. J Enzyme Inhib Med Chem [Internet]. 2020 Jan 1;35(1):692–701. Available from: <URL>.